Supplemental Results

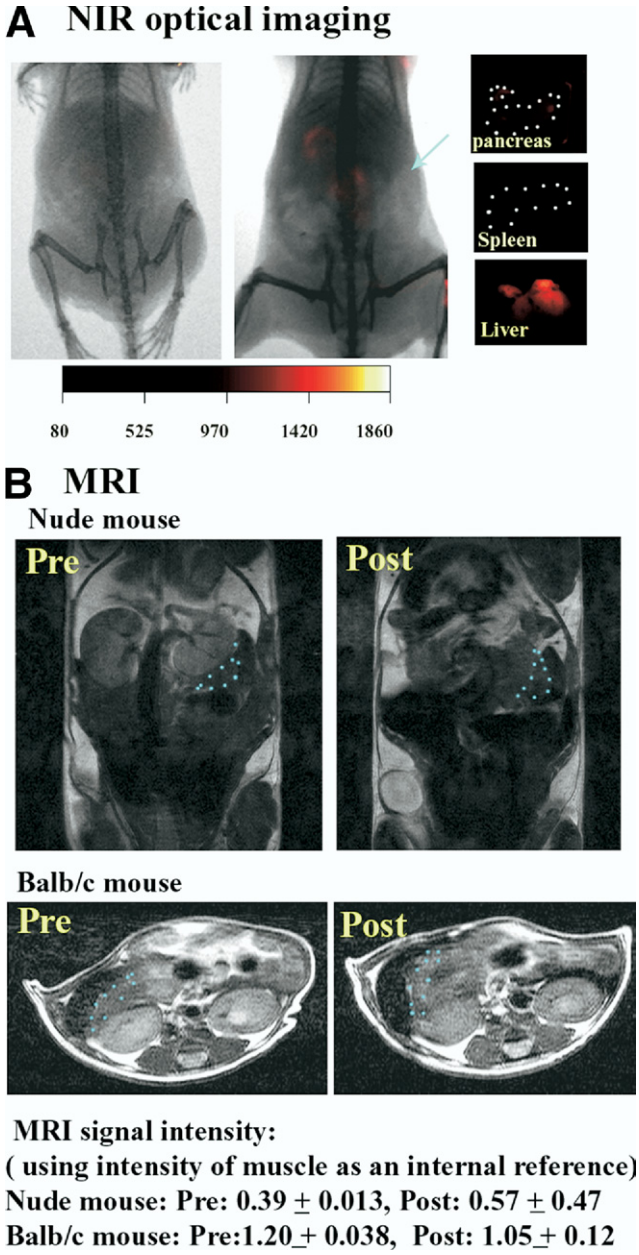
Examination of Target Specificity of ATF-IO Nanoparticles in a Chronic Pancreatitis Mouse Model

To determine the target specificity of ATF-IO nanoparticles to pancreatic cancer, we have established a chronic pancreatitis model by surgically ligating the pancreatic duct at the pancreatic tail of the mice.[1] Our results showed that systemic administration of Cy5.5-ATF-IO nanoparticles did not produce optical signals or MRI T_2 contrast decreases in the pancreas of the mice that received the surgical procedure for 2 months (Supplementary Figure 1*A* and *B*). Only a weak imaging signal was detected in the liver of the mice. We used both nude and Balb/c mice to evaluate the imaging specificity of the targeted nanoparticles because an immunocompetent BALB/c mouse may produce stronger inflammatory response in the pancreas compared with a nude mouse.

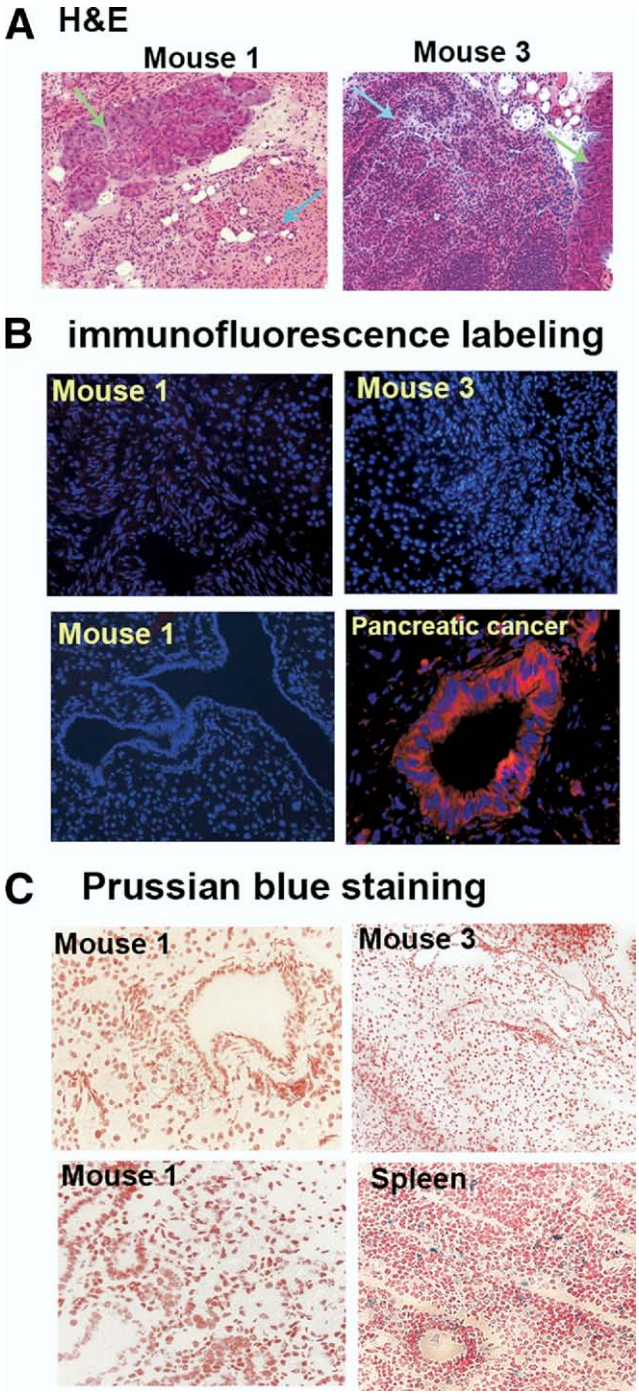
Examination of the pancreas of the mice that received double ligation of the pancreatic duct revealed pathologic and histologic characteristics of chronic pancreatitis, such as destruction of acinus, fibrosis, and infiltration of inflammatory cells (Supplementary Figure 2*A*). Importantly, immunofluorescence labeling showed that the level of uPAR expression was not up-regulated in the pancreatitis tissues (Supplementary Figure 2*B*). As a positive control, a high level of uPAR was detected in a human pancreatic cancer tissue. Prussian blue staining showed that ATF-IO nanoparticles were not accumulated in the pancreas containing chronic pancreatitis lesions (Supplementary Figure 2*C*). However, positive Prussian blue staining was found in the spleen of the mouse.

Reference

1. Sakaguchi Y, Inaba M, Kusafuka K, et al. Establishment of animal models for three types of pancreatitis and analyses of regeneration mechanisms. Pancreas 2006;33:371–381.



Supplementary Figure 1. In vivo optical and MR imaging of the mice with chronic pancreatitis after systemic delivery of Cy5.5-ATF-IO nanoparticles. Following ligation of the pancreatic duct for 2 months, the mice received a tail vein injection of 200 pmol of Cy5.5-ATF-IO nanoparticles for 24 to 48 hours. (*A*) NIR optical imaging was performed using the Kodak In Vivo Imaging System. Optical signal was not detected in the pancreatic area (*arrow*). Some weak signals were detected in the right and middle of the abdominal cavity. Ex vivo tissue imaging further confirmed the absence of the optical signal in the pancreas. However, weak NIR signal was found in the liver (*A*) and intestine (data not shown). (*B*) In vivo MRI of the mice with chronic pancreatitis. MRI was performed before (Pre) and 24 hours after the nanoparticle injection (Post). *Blue dotted areas* are locations of the pancreas. The mean contrast intensity of 3 randomly selected regions within the pancreas was measured and calculated from a nude or BALB/c mouse. There was no significant decrease in MRI contrast in both mice after ATF-IO nanoparticle injection.



Supplementary Figure 2. Histologic analysis of the pancreatic tissues. (*A*) H&E staining of frozen tissue sections obtained from the mice that received double ligation of the pancreatic duct for 2 months. Pathologic characteristics of chronic pancreatitis were found in the tissues, such as fibrosis, destruction of acinus, dilated and proliferation of the pancreatic ducts, and infiltration of inflammatory cells. Mouse 1, nude mouse; mouse 3, Balb/c mouse. *Green arrows*, pancreatic acinus; *blue arrows*, histologic changes of chronic pancreatitis. (*B*) Frozen sections of the pancreas obtained from mice with chronic pancreatitis or a positive control human pancreatic cancer tissue were labeled with a polyclonal anti-uPAR antibody that reacts with both human and mouse uPAR, followed by fluorescence dye-labeled secondary antibody. uPAR expression was detected in human pancreatic cancer but not in the pancreas of the mice with chronic pancreatitis. *Red*, uPAR; *blue*, Hoechst 33342 background staining. (*C*) Prussian blue staining. IO nanoparticles were not detected in the pancreas with chronic pancreatitis. However, the IO nanoparticles were seen in the spleen of the mice. *Red*, nuclear fast red background staining.